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Claims 1, 3 and 5 have been amended to recite that the claimed variants are phenotypically silent amino acid substitution variants. Support for these amendments can be found in the specification at, *inter alia*, page 7, line 14 through page 8, line 20. New claims 7-9 have been added. The new claims recite that variants are conservative amino acid substitution variants. Support for the amendments can be found at page 7, line 14 through page 8, line 20.

No new matter is added by these amendments. Applicants respectfully request entry of these amendments.

Objections to the Specification

The specification has been objected to for the use of unitalicized term "Ehrlichia". The specification has been amended to italicize all occurrences of this word.

The specification has also been objected to as containing the use of trademarks throughout the specification. The Office Action requests correction. The MPEP states that the use of trademarks having definite meanings is permissible in patent applications as long as the proprietary nature of the marks are respected. See MPEP §608.01(v). The MPEP further states that trademarks should be identified by capitalizing each letter of the mark. The specification refers to the SNAP® trademark in all capital letters and also provides a registration mark where the trademark is used. As such, the proprietary nature of the mark is respected. Furthermore, the meaning of the term is definite as used in the specification. The specification teaches that:

> A preferred assay of the invention is the reversible flow chromatographic binding assay, for example a SNAP® assay. *See* U.S. Pat. No. 5,726,010. See page 13, lines 14-16.

The use of trademarks in the instant specification is proper. Applicants respectfully request withdrawal of the objections to the application.

Rejection of Claims 1-6 Under 35 U.S.C. §112, first paragraph

Claims 1-6 stand rejected under 35 U.S.C. §112, first paragraph as allegedly lacking written description. Applicants respectfully traverse the rejection.

The Office Action asserts that variants of SEQ ID NO:2 are not adequately described in the specification. The standard for written description is whether the specification conveys with reasonable clarity to those skilled in the art that, as of the filing date sought, the Applicant was in possession of the invention as now claimed. *See Vas-Cath, Inc. v. Mahurkar*, 19 U.S.P.Q.2d 1111, 1117 (Fed. Cir. 1991). An Applicant shows possession of the claimed invention with all of its limitations using such descriptive words, structures, figures, diagrams, and formulas that fully set forth the claimed invention. See *id.* Furthermore:

> conception [and therefore possession] of a chemical compound requires that the inventor be able to define it so as to distinguish it from other materials, and to describe how to obtain it. Conception does not occur unless one has a mental picture of the structure of the chemical, or is able to define it by its method of preparation, its physical or chemical properties, or whatever characteristics sufficiently distinguish it. It is not sufficient to define it solely by its principal biological property, e.g., encoding human erythropoietin, because an alleged conception having no more specificity than that is simply a wish to know the identity of any material with that biological property. *See Amgen, Inc. v. Chugai Pharmaceutical Co.*, 18 U.S.P.Q.2d 1016, 1021 (Fed. Cir. 1991).

Claims 1, 3, and 5 have been amended to recite that the variant of SEQ ID NO:2 is a phenotypically silent amino acid substitution variant. Additionally, new claims 7-9 have been added. These claims recite that the variant of SEQ ID NO:2 is a conservative amino acid substitution variant. Given the specification, one of skill in the art would

recognize that the Applicants were in possession of an isolated polypeptide of SEQ ID NO:2, phenotypically silent amino acid substitution variants of SEQ ID NO:2, and conservative amino acid substitution variants of SEQ ID NO:2

The specification teaches that the invention the variants are phenotypically silent amino acid substitutions and/or conservative amino acid substitutions and provides detailed guidance on how to construct such variants. See, page 7, line 14 through page 8, line 20. *See also*, Bowie, et al., *Science*, 247:1306(1990) (copy attached) (teaching methods of construction of variants and the tolerance of protein sequences of substitutions).

The specification teaches that: "[p]olypeptides that do not comprise 100% identity to a polypeptide sequence shown in SEQ ID NOs:1-7 are considered 'variants'" and that "the invention provides polypeptides having at least 85% identity, more preferably at least 90% identity, and still more preferably at least 96%, 97%, 98%, or 99% identity to a polypeptide sequence shown in SEQ ID NOs:1-7." See page 5, lines 7-14.

The specification goes on to define the meaning of "identity" and explains that sequences are aligned for identity calculations using a mathematical algorithm. See page 6, line 3 through page 7, line 5. The specification furthermore provides guidance concerning how to make phenotypically silent amino acid substitutions. See page 7, line 14 through page 8, line 20.

The specification also specifies that :

> Polypeptides of the invention specifically bind to an anti-*Ehrlichia* antibody. In this context "specifically binds" means that the polypeptide recognizes and binds to an anti-*Ehrlichia* antibody, but does not substantially recognize and bind other molecules in a test sample. See page 9, lines 8-11.

The specification also teaches how to screen a variant polypeptide to determine whether it binds to an anti-*Ehrilichia* antibody. See, e.g., page 18, line 19 through page 19, line 13.

Therefore, the specification teaches that a phenotypically silent amino acid substitution variant or conservative amino acid substitution variant polypeptide of the invention has at least 85% identity, more preferably at least 90% identity, and still more preferably at least 96%, 97%, 98%, or 99% identity to a polypeptide sequence shown in SEQ ID NO:2, and that it specifically binds to an anti-*Ehrlichia* antibody. One of skill in the art would recognize that variations can be made in a polypeptide shown in SEQ ID NO:2 without affecting antigenicity. See specification page 8, lines 9-20 (teaching that proteins are surprisingly tolerant of amino acid substitutions and providing guidance to the types of amino acid substitutions that are well tolerated). Furthermore, one of skill in the art would recognize that the Applicants were in possession of variant polypeptides having a certain percentage sequence identity to SEQ ID NO:2, comprising either phenotypically silent amino acid substitutions or conservative amino acid substitutions, and that also specifically bind an anti-*Ehrlichia* antibody.

Applicants demonstrated possession of phenotypically silent amino acid substitution variants and conservative amino acid substitution variants of SEQ ID NO:2. The physical and chemical properties of such variants have been described such that one of skill in the art would recognize that the applicants were in possession of the claimed

variants when the application was filed. For example, the specification teaches that the variants are phenotypically silent amino acid substitutions or conservative amino acid substitutions, that the variants have at least 85% identity to SEQ ID NO:2, that the variants specifically bind to an anti-*Ehrlichia* antibody, and the specification teaches how to make and test the claimed variants. Therefore, the claimed variants have adequate written description in the specification.

Applicants respectfully request withdrawal of the rejection.

Rejection of Claims 1-6 Under 35 U.S.C. §112, first paragraph

Claims 1-6 stand rejected under 35 U.S.C. §112, first paragraph as allegedly lacking enablement. Applicants respectfully traverse the rejection.

The Office Action asserts that the specification does not provide enablement for a composition or an article of manufacture that comprise variants of SEQ ID NO:2. The claims have been amended to recite that the variants are either phenotypically silent amino acid substitution variants of SEQ ID NO:2 or conservative amino acid substitution variants of SEQ ID NO:2. Under 35 U. S. C. § 112, all that is required is that the specification describe the invention in such terms as to enable a person skilled in the art to make and use the invention. Thus, the specification must teach one skilled in the art how to make and use a variant of a polypeptide shown in SEQ ID NO:2. The test of enablement is whether one reasonably skilled in the art (1) could make and use the invention (2) from the disclosures in the patent coupled with information known in the art (3) without undue experimentation. *In re Wands*, 858 F.2d 731 (Fed. Cir. 1988); *United States v. Telectronics, Inc.*, 857 F.2d 778 (Fed. Cir. 1988); M.P.E.P. § 2164.01. "The determination of what constitutes undue experimentation is a given case requires the

application of a standard of reasonableness, having due regard of the nature of the invention and the state of the art." *In re Wa*nds, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988) (citing *Ansul Co. v. Uniroyal, Inc.*, 169 U.S.P.Q. 759, 762-63 (2d Cir. 1971).

The specification teaches that a variant polypeptide of the invention has at least 85% identity, more preferably at least 90% identity, and still more preferably at least 96%, 97%, 98%, or 99% identity to a polypeptide sequence shown in SEQ ID NO:2 and it specifically binds to an anti-*Ehrlichia* antibody. One of skill in the art could easily design and make a polypeptide that falls within the given percentage sequence identity and screen it for specific binding to an anti-*Ehrlichia* antibody. For example, in the case of SEQ ID NO:2, which is an about 20 amino acid long polypeptide, a variant polypeptide having 85% identity would have only about 3 substituted amino acids. According to the claims these substituted amino acids are phenotypically silent amino acid substitutions or conservative amino acid substitutions. One of skill in the art, given the specification, could easily design and make such a phenotypically silent amino acid substitution variant polypeptide or conservative amino acid substitution variant polypeptide given SEQ ID NO:2. *See e.g.,* page 7, line 14 through page 8, line 20.

One of skill in the art can clearly make a polypeptide once the sequence was designed. Additionally, the specification teaches that a polypeptide can be made by, for example, conventional peptide synthesis or by recombinant techniques. See page 10, lines 6-13. One of skill in the art could then screen a variant polypeptide for binding to an anti-*Ehrlichia* antibody by the methods described in Example 1.

The law is well settled that the test of enablement "is not merely quantitative, since a considerable amount of experimentation is permissible, if it merely routine, or if

the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *In re Wa*nds, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988) (citing *Ansul Co. v. Uniroyal, Inc.*, 169 U.S.P.Q. 759, 762-63 (2d Cir. 1971). One of skill in the art understands the meaning of sequence identity and most certainly could design and make a polypeptide sequence that has 85% or more sequence identity to SEQ ID NO:2 using only routine experimentation. Once one of skill in the art had designed and made a phenotypically silent amino acid substitution variant polypeptide or conservative amino acid variant polypeptide of the invention, they could use only routine screening to identify whether the polypeptide specifically binds to an anti-*Ehrlichia* antibody. Therefore, even though it could conceivably take a considerable amount of experimentation to design and make a phenotypically silent amino acid substitution variant polypeptide or conservative amino acid variant polypeptide of the invention, such design and manufacture requires only routine experimentation that is well-known and understood to one of skill in the art. Additionally, the specification provides direction to guide one of skill in the art to the experimentation that is necessary to design, make and screen a phenotypically silent amino acid substitution variant polypeptide or conservative amino acid variant polypeptide of the invention.

Finally, the specification teaches that a phenotypically silent amino acid substitution variant polypeptide or conservative amino acid variant polypeptide can be used to detect the presence of anti-*Ehrlichia* antibodies. See page 11, line 22 through page 17, line 9. Therefore, one of skill in the art, given the specification could make and use the phenotypically silent amino acid substitution variant polypeptide or conservative amino acid variant polypeptide of the invention without undue experimentation.

Applicants respectfully request withdrawal of the rejection.

Rejection of Claims 3 and 5 Under 35 U.S.C. §112, second paragraph

Claims 3 and 5 stand rejected under 35 U.S.C. §112, second paragraph as allegedly indefinate. Applicants respectfully traverse the rejection.

Under section 112, second paragraph "distinctly claim" means that claims must have "a clear and definite meaning when construed in light of the complete patent document." *See Standard Oil Co. v. American Cyanamide Co.*, 227 U.S.P.Q. 293, 296 (Fed. Cir. 1985). The test of definiteness is "whether those skilled in the art would understand what is claimed when the claim is read in light of the specification." *Orthokinetics, Inc. v. Safety Travel Chairs, Inc.*, 1 U.S.P.Q.2d 1081, 1088 (Fed. Cir. 1986). A claim need not describe the invention, such description being the role of the disclosure portion of the specification, not the role of the claims. *Id.*

The Office Action asserts that claim 3 is indefinate because it recites "a polypeptide shown in SEQ ID NO:2" and alleges that it is unclear as to what the Applicant is referring.

Claim 3 recites "a polypeptide shown in SEQ ID NO:2." One of skill in the art understands that a polypeptide is a chain of amino acids. The specification teaches that SEQ ID NO:2 is NTTTGVFGLKQDWDGATIKD. See Table 1, page 6. One of skill in the art would clearly understand that "a polypeptide shown in SEQ ID NO:2" refers to the following polypeptide: NTTTGVFGLKQDWDGATIKD. As such, claim 3 is definite when read in light of the specification.

The Office Action asserts that claim 5 is indefinate because it recites "under conditions." The Office Action appears to not understand to what "under conditions"

refers. The phrase of claim 5, however, reads "under conditions that allow polypeptide/antibody complexes to form." Therefore, the conditions to which claim 5 refers are conditions under which polypeptide/antibody complexes can form. One of skill in the art would understand what is claimed when the claim is read in light of the specification. That is, one of skill in the art would understand the conditions necessary to allow polypeptide/antibody complexes to form in an assay. Such assays are very well known in the art and are described in the specification at, *inter alia,* Example 1.

The Office Action further asserts that claim 5 is indefinite because it is unclear whether the claim is reciting a product or a process. Claim 5 has been amended to clarify that the process steps recited in the claim are indicated on the label of the article of manufacture.

Claims 3 and 5 are therefore definite and Applicants respectfully request withdrawal of the rejection.

Rejection of Claims 1-6 Under 35 U.S.C. §102(a)

Claims 1-6 stand rejected under 35 U.S.C. §102(a) as allegedly anticipated by Waner *et al.* Applicants respectfully traverse the rejection.

Anticipation under 35 U.S.C. §102 requires the presence in a single prior art disclosure of each and every element of a claimed invention. *Lewmar Marine Inc. v. Barient Inc.,* 3 U.S.P.Q.2d 1766, 1767 (Fed. Cir. 1987).

The claims recite compositions of matter and articles of manufacture that include an isolated polypeptide shown in SEQ ID NO:2, a phenotypically silent amino acid substitution variant of SEQ ID NO:2, and a conservative amino acid substitution variant of SEQ ID NO:2.

The Office Action asserts that Waner teaches a commercial ELISA for *E. canis* and that the polypeptides recited in the instant invention would be inherent in the teachings of Waner.

Waner does not teach or suggest the use of distinct *E. canis* polypeptides as shown in SEQ ID NO:2. That is, Waner does not teach or suggest an about 20 amino acid polypeptide of SEQ ID NO:2 or the specified variants of SEQ ID NO:2. Instead, Waner teaches an IFA for *Ehrlichia canis* that uses DH82 cells that are heavily infected with *E. canis* as an antigen. See page 240, second column, last paragraph. Waner also teaches an ELISA for *E. canis* that uses an *E. canis* antigen derived from mouse J774.A1-infected cells. See page 241, first column, first full paragraph. Waner, therefore, teaches entire cells or whole proteins as assay antigens. As such, Waner can not teach, suggest, or inherently disclose the specific, individual polypeptides shown in SEQ ID NO:2 or the specified variants of SEQ ID NO:2. Furthermore, Waner does not identify the polypeptide fragments to be of any particular diagnostic use. There is no teaching in Waner, directly or inherently, that would direct one of skill in the art to the particular defined sequences of SEQ ID NO:2 or the specified variants for any reason. Warner does not teach or suggest that polypeptides of SEQ ID NO:2 would be useful as individual polypeptides apart from entire *E. canis* infected cells or entire proteins. Warner provides no recognition or suggestion that the distinct polypeptides shown in SEQ ID NO:2 or the specified variants or any other polypeptide fragments would be of diagnostic use.

Importantly, the specification teaches that:

> Indirect immunofluorescense assays (IFA) and enzyme-linked immunosorbent assays (ELISA) are frequently used as aids in the diagnosis of these diseases. These assays measure or otherwise detect the binding of anti-*Ehrlichia* antibodies from a patient's blood, plasma, or

> serum to infected cells, cell lysates, or purified *Ehrlichia* proteins. However, currently known assays for detecting anti-*Ehrlichia* antibodies or fragments thereof are severely limited in usefulness because of sensitivity and specificity issues directly related to the impure nature of the *Ehrlichia* antigen used in these tests. See page 2, line 21 through page 3, line 2 (emphasis added).

The instant invention provides highly purified reagents for the detection *Ehrlichia*, that is, polypeptides of about 20 amino acids, whereas Waner teaches the use of reagents comprising whole infected cells or whole *E. canis* proteins derived from infected cells. The Waner reagents are impure reagents, which the instant specification teaches are of limited usefulness due to the sensitivity and specificity issues. For instance, Example 1 demonstrates that assays that use a synthetic peptide were more sensitive and specific than assays that use native *E. canis* antigens, i.e., partially purified *E. canis* antigens

Additionally, the Office Action is relying on an inherency theory to sustain this anticipation rejection. However, where an Examiner relies upon an inherency theory:

> the Examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art. See *Ex parte Levy*, 17 U.S.P.Q. 1461,1464 (Bd. Pat. App. & Inter. 1990) (emphasis in original); M.P.E.P. §2112.

The Office Action has provided no reasoning or evidence tending to show inherency in the instant case. Rather the Office Action relies upon the statement the article of manufacture "appears" to be the same as the claimed invention and baldly asserts that the claimed polypeptides are "inherent in the teachings of the prior art" without providing any reasoning or evidence why the claimed about 20 amino acid polypeptides as shown in SEQ ID NO:2 would be present in Waner. The Office Action

asserts that it does not have the facilities for examining and comparing the claimed compositions and articles with the device of the prior art and asserts that the burden is on the applicant to show a novel or unobvious difference between the claimed product ant the product of the prior art. However, the initial burden of establishing a *prima facia* basis to deny patentablity to a claimed invention rests upon the examiner. See *Ex parte Levy* at 1463-1464. The Examiner has not discharged this initial burden in this case.

Furthermore, the Office Action has provided no extrinsic evidence, other than a bald assertion, to support the alleged inherency finding.

> To establish inherency, the extrinsic evidence "must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill." Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient. *See In re Robertson*, 49 U.S.P.Q.2d 1949, 1950-1951 (Fed. Cir. 1999) (citations omitted); M.P.E.P §2112.

The Office Action has provided no extrinsic evidence that the claimed about 20 amino acids polypeptides are present in the cited reference.

Waner does not anticipate claims 1-6 because Waner does not teach, suggest, or inherently disclose each and every element of claims 1-6. Applicants respectfully request withdrawal of the rejection.

Rejection of Claims 1-6 Under 35 U.S.C. §102(b)

Claims 1-6 stand rejected under 35 U.S.C. §102(b) as allegedly anticipated by Cadman *et al.* Applicants respectfully request withdrawal of the rejection.

Similar to the Waner rejection above, the Office Action asserts that the polypeptide compositions and article of manufacture of the invention are inherently present in the assays disclosed in Cadman.

Cadman does not teach or suggest the use of distinct *E. canis* polypeptides as shown in SEQ ID NO:2 and the claimed specified variants. That is, Cadman does not teach or suggest an about 20 amino acid polypeptide of SEQ ID NO:2 or the specified variants. Cadman teaches an IFA for *Ehrlichia canis* that uses DH82 cells which are heavily infected with *E. canis* as an antigen. See Cadman, first column, fourth paragraph. Cadman also teaches a dot-blot enzyme linked immunoassay (DBELIA) for *E. canis* that uses an *E. canis* antigen purified from infected DH82 cells. See Cadman, first column, fifth paragraph. As such, Cadman teaches the use of whole *E. canis* infected cells or whole proteins purified from E. canis infected cells in the disclosed assays. Therefore, Cadman does not teach, suggest, or inherently disclose the specific, individual polypeptides shown in SEQ ID NO:2 and does not identify the polypeptide fragments to be of any particular diagnostic use. There is no teaching in Cadman, directly or inherently, that would direct one of skill in the art to the particular, defined sequences of SEQ ID NO:2 or specified variants for any reason. Cadman does not teach or suggest that polypeptides of SEQ ID NO:2 or specified variants would be useful as individual polypeptides apart from entire *E. canis* infected cells or entire proteins. Cadman provides no recognition or suggestion that the distinct polypeptides shown in SEQ ID NO:2, specified variants, or any other polypeptide fragments would be of diagnostic use.

Additionally, Cadman teaches the use of impure reagents, i.e., whole *E. canis* infected cells or whole proteins derived from infected cells. The instant invention, however, provides highly purified reagents that are much more sensitive and specific. See e.g., specification page 2, line 21 through page 3, line 2. For instance, Example 1

demonstrates that assays that use a synthetic peptide were more sensitive and specific than assays that use native *E. canis* antigens, i.e.; partially purified *E. canis* antigens.

Additionally, as discussed above, for Warner, the Office Action has not provided a *prima facia* basis to deny patentablity because the Office Action has not provided a basis in fact, technical reasoning, and/or extrinsic evidence to demonstrate that the claimed polypeptides are present in the Cadman devices.

Cadman does not teach each and every element of the claimed invention and therefore does not anticipate the claimed invention. Applicants respectfully request withdrawal of the rejection.

Rejection of Claims 1-6 Under 35 U.S.C. §102(b)

Claims 1-6 stand rejected under 35 U.S.C. §102(b) as allegedly anticipated by Zhi. Applicants respectfully traverse the rejection.

The Office Action asserts that the polypeptides and articles of manufacture of the invention are inherently present in the assays reported in Zhi. Initially, Zhi teaches assays for the detection of Human Granulocytic Ehrlichiosis Agent (HGE). HGE is closely related to or identical to *E. equi* and *E. phagocytophilia*. See CDC Publication, "Human Ehrlichioisis in the United States;" Dumler *et al.*, Int. J. Syst. Evol. Microbiol. 51:2145 (2001) (abstract) (copies attached). Therefore, Zhi does not teach or suggest *E. canis* antigens, proteins or polypeptides or articles of manufacture containing *E. canis* polypeptides, including a polypeptide shown in SEQ ID NO:2 or its specified variants. However, in the event that the HGE taught in Zhi could be considered to be *E. canis*, Zhi would still not teach or suggest each and every element of claims 1-6.

Zhi teaches Western immunoblot analysis and dot immunoblot assays for HGE that uses HGE rP44, a 35kDa fusion protein, or purified HGE organisms as assay antigens. See page 1668, first column, first and second full paragraphs; page 1668, second column, first full paragraph.

Zhi does not teach or suggest the use of distinct *E. canis* polypeptides as shown in SEQ ID NO:2 pr the specified variants. Rather, Zhi teaches the use of HGE rP44 or purified HGE organisms in the disclosed assays. Therefore, Zhi does not teach, suggest, or inherently disclose the specific, individual polypeptide shown in SEQ ID NO:2, or its specified variants and does not identify the polypeptide fragment to be of any particular diagnostic use. There is no teaching in Zhi, directly or inherently, that would direct one of skill in the art to the particular, defined sequences of SEQ ID NOs:2 for any reason. Zhi does not teach or suggest that SEQ ID NOs:2 is a sequence that would be useful as individual peptides apart from entire HGE organisms or HGE rP44. Zhi provides no recognition or suggestion that the distinct polypeptide shown in SEQ ID NO:2 or its specified variants would be of diagnostic use.

Additionally, as discussed above, for Warner, the Office Action has not provided a *prima facia* basis to deny patentablity because the Office Action has not provided a basis in fact, technical reasoning and/or extrinsic evidence to demonstrate that the claimed polypeptides are present in the Zhi devices.

Zhi does not anticipate claims 1-6 because Zhi does not teach, suggest, or inherently disclose each and every element of claims 1-6. Applicants respectfully request withdrawal of the rejection.

Rejection of Claims 1-6 Under 35 U.S.C. §102(b)

Claims 1-6 stand rejected under 35 U.S.C. §102(b) as allegedly anticipated by Rikihisa et al. (WO 99/13720). Applicants respectfully traverse the rejection.

The Office Action asserts that Rikihisa teaches the polypeptide of SEQ ID NO:2, and that an article of manufacture is inherent in the teachings of Rikihisa.

Rikihisa does not teach or suggest the use of distinct *E. canis* polypeptides as shown in SEQ ID NO:2 or its specified variants. That is, Rikihisa does not teach or suggest an about 20 amino acid polypeptide of SEQ ID NO:2 or its specified variants. Rather Rikihisa teaches a 288 amino acid sequence. Therefore, Rikihisa does not teach, suggest, or inherently disclose the specific, individual polypeptides shown in SEQ ID NO:2 or its specified variants and does not identify the polypeptide fragments to be of any particular diagnostic use. There is no teaching in Rikihisa, directly or inherently, that would direct one of skill in the art to the particular, defined sequences of SEQ ID NO:2 or its specified variants for any reason. Rikihisa does not teach or suggest that polypeptides of SEQ ID NO:2 would be useful as individual polypeptides apart from the entire *E. canis* P30 protein. Rikihisa provides no recognition or suggestion that the distinct polypeptides shown in SEQ ID NO:2 or its specified variants or any other polypeptide fragments would be of diagnostic use.

Additionally, as discussed above, for Warner, the Office Action has not provided a *prima facia* basis to deny patentablity because the Office Action has not provided a basis in fact, technical reasoning, and/or extrinsic evidence to demonstrate that the claimed polypeptides are present in the Rikihisa devices.

Rikihisa does not teach each and every element of the claimed invention and therefore does not anticipate the claimed invention. Applicants respectfully request withdrawal of the rejection.

Respectfully submitted,

Date: 11/4/02 by: _____

Lisa M.W. Hillman
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Appendix A

Marked-Up Version of the Amendments to Show Changes Made

IN THE CLAIMS:

1. (Amended) A composition of matter [comprising] consisting essentially of an isolated polypeptide shown in SEQ ID NO:2 [or variants thereof] or a phenotypically silent amino acid substitution variant thereof.

3. (Amended) An article of manufacture comprising packaging material and, contained within the packaging material, a polypeptide shown in SEQ ID NO:2 [or variants thereof] or a phenotypically silent amino acid substitution variant thereof.

4. (Amended) The article of manufacture of claim 3 wherein the packaging material comprises a label that indicates that the polypeptide can be used for the identification of [Ehrlichia] *Ehrlichia* infection in a mammal.

5. (Amended) The article of manufacture of claim 4, wherein the label indicates that identification of an [Ehrlichia] *Ehrlichia* infection is done using a method of detecting presence of antibodies to [Ehrlichia] *Ehrlichia* comprising:

(a) contacting a polypeptide shown in SEQ ID NO:2 or a phenotypically silent amino acid substitution variant of the polypeptide shown in SEQ ID NO:2, [or variants thereof,] with a test sample suspected of comprising antibodies to [Ehrlichia] *Ehrlichia*, under conditions that allow polypeptide/antibody complexes to form; and

(b) detecting polypeptide/antibody complexes;

wherein the detection of polypeptide/antibody complexes is an indication that an [Ehrlichia] *Ehrlichia* infection is present.

6. (Amended) The article of manufacture of claim 4, wherein the [Ehrlichia] *Ehrlichia* infection is caused by *Ehrlichia canis* or *Ehrlichia chaffeensis.*

7. (New) A composition of matter consisting essentially of an isolated polypeptide shown in SEQ ID NO:2 or a conservative amino acid substitution variant thereof.

8. (New) An article of manufacture comprising packaging material and, contained within the packaging material, a polypeptide shown in SEQ ID NO:2 or a conservative amino acid substitution variant thereof.

9. (New) The article of manufacture of claim 4, wherein the label indicates that identification of an *Ehrlichia* infection is done using a method of detecting presence of antibodies to *Ehrlichia* comprising:

(a) contacting a polypeptide shown in SEQ ID NO:2 or a conservative amino acid substitution variant of the polypeptide shown in SEQ ID NO:2, with a test sample suspected of comprising antibodies to *Ehrlichia*, under conditions that allow polypeptide/antibody complexes to form; and

(b) detecting polypeptide/antibody complexes;

wherein the detection of polypeptide/antibody complexes is an indication that an *Ehrlichia* infection is present.

IN THE SPECIFICATION:

Please replace the second full paragraph of page 2, with the following paragraph:

The [Ehrilichia] *Ehrlichia* are obligate intracellular pathogens that infect circulating lymphocytes in mammalian hosts. *Ehrlichia canis* and *Ehrlichia chaffeensis*

are members of the same sub-genus group that infect canines and humans and cause canine monocytic ehrlichiosis (CME) and human monocytic ehrlichiosis (HME), respectively. The canine disease is characterized by fever, lymphadenopathy, weight loss, and pancytopenia. In humans the disease is characterized by fever, headache, mylagia, and leukopenia. Early detection and treatment are important for treating both canine and human ehrlichiosis.

Please replace the third full paragraph of page 2 with the following paragraph:

Indirect immunofluorescense assays (IFA) and enzyme-linked immunosorbent assays (ELISA) are frequently used as aids in the diagnosis of these diseases. These assays measure or otherwise detect the binding of [anti-Ehrlichia] anti-*Ehrlichia* antibodies from a patient's blood, plasma, or serum to infected cells, cell lysates, or purified [Ehrlichia] *Ehrlichia* proteins. However, currently known assays for detecting [anti-Ehrlichia] anti-*Ehrlichia* antibodies or fragments thereof are severely limited in usefulness because of sensitivity and specificity issues directly related to the impure nature of the [Ehrlichia] *Ehrlichia* antigen used in these tests. Highly purified reagents are needed to construct more accurate assays.

Please replace the forth full paragraph of page 3 with the following paragraph:

Still another embodiment of the invention provides a method of detecting the presence of antibodies to [Ehrlichia] *Ehrlichia*. The method comprises contacting one or more polypeptides selected from the group consisting of the polypeptides shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and variants thereof, with a test sample suspected of comprising antibodies to [Ehrlichia] *Ehrlichia*, under conditions that allow polypeptide/antibody

complexes to form. The polypeptide/antibody complexes are detected. The detection of polypeptide/antibody complexes is an indication that antibodies to [Ehrlichia] *Ehrlichia* are present in the test sample.

Please replace the first full paragraph of page 4 with the following paragraph:

Yet another embodiment of the invention provides a device containing one or more polypeptides selected from the group consisting of the polypeptides shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and variants thereof, and instructions for use of the one or more polypeptides for the identification of an [Ehrlichia] *Ehrlichia* infection in a mammal.

Please replace the second full paragraph of page 4 with the following paragraph:

Still another embodiment of the invention provides an article of manufacture comprising packaging material and, contained within the packaging material, one or more polypeptides selected from the group consisting of the polypeptides shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and variants thereof. The packaging material comprises a label that indicates that the one or more polypeptides can be used for the identification of [Ehrlichia] *Ehrlichia* infection in a mammal.

Please replace the third full paragraph of page 4 with the following paragraph:

Even another embodiment of the invention provides a method of diagnosing an [Ehrlichia] *Ehrlichia* infection in a mammal. The method comprises obtaining a biological sample from a mammal suspected of having an [Ehrlichia] *Ehrlichia* infection, and contacting one or more polypeptides selected from the group consisting of the polypeptides shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ

ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and variants thereof, with the biological sample under conditions that allow polypeptide/antibody complexes to form. Polypeptide/antibody complexes are detected, wherein the detection of polypeptide/antibody complexes is an indication that the mammal has an [Ehrlichia] *Ehrlichia* infection.

Please replace the first full paragraph of page 5 with the following paragraph:

The invention therefore provides highly purified polypeptides and antibodies for use in accurate assays for the detection of [Ehrlichia] *Ehrlichia* antibodies and antibody fragments.

Please replace the third full paragraph of page 7 with the following paragraph:

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, the amino acid positions which have been conserved between species can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions in which substitutions have been tolerated by natural selection indicate positions which are not critical for protein function. Thus, positions tolerating amino acid substitution may be modified while still maintaining specific binding activity of the polypeptide to [anti-Ehrlichia] anti-*Ehrlichia* antibodies or antibody fragments.

Please replace the first full paragraph of page 8 with the following paragraph:

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site-directed mutagenesis or alanine-scanning mutagenesis (the introduction of

single alanine mutations at every residue in the molecule) can be used (Cunningham *et al.*, *Science,* 244:1081-1085 (1989)). The resulting mutant molecules can then be tested for specific binding to [anti-Ehrlichia] anti-*Ehrlichia* antibodies or antibody fragments.

Please replace the first full paragraph of page 9 with the following paragraph:

Polypeptides of the invention specifically bind to an [anti-Ehrlichia] anti-*Ehrlichia* antibody. In this context "specifically binds" means that the polypeptide recognizes and binds to an [anti-Ehrlichia] anti-*Ehrlichia* antibody, but does not substantially recognize and bind other molecules in a test sample.

Please replace the second full paragraph of page 9 with the following paragraph:

Polypeptides of the invention comprise at least one epitope that is recognized by an [anti-Ehrlichia] anti-*Ehrlichia* antibody. An epitope is an antigenic determinant of a polypeptide. An epitope can be a linear, sequential epitope or a conformational epitope. Epitopes within a polypeptide of the invention can be identified by several methods. *See, e.g.,* U.S. Patent No. 4,554,101; Jameson & Wolf, *CABIOS* 4:181-186 (1988). For example, a polypeptide of the invention can be isolated and screened. A series of short peptides, which together span the entire polypeptide sequence, can be prepared by proteolytic cleavage. By starting with, for example, 20-mer polypeptide fragments, each fragment can be tested for the presence of epitopes recognized in, for example, an enzyme-linked immunosorbent assay (ELISA). In an ELISA assay a polypeptide, such as a 20-mer polypeptide fragment, is attached to a solid support, such as the wells of a plastic multi-well plate. A population of antibodies are labeled, added to the solid support and allowed to bind to the unlabeled antigen, under conditions where non-specific adsorbtion is blocked, and any unbound antibody and other proteins are washed

away. Antibody binding is detected by, for example, a reaction that converts a colorless indicator reagent into a colored reaction product. Progressively smaller and overlapping fragments can then be tested from an identified 20-mer to map the epitope of interest.

Please replace the first full paragraph of page 10 with the following paragraph:

Preferably, a polypeptide of the invention is synthesized using conventional peptide sythesizers, which are well known in the art. A polypeptide of the invention can also be produced recombinantly. A polynucleotide encoding an [Ehrlichia] *Ehrlichia* polypeptide can be introduced into an expression vector that can be expressed in a suitable expression system using techniques well known in the art. A variety of bacterial, yeast, plant, mammalian, and insect expression systems are available in the art and any such expression system can be used. Optionally, a polynucleotide encoding an [Ehrlichia] *Ehrlichia* polypeptide can be translated in a cell-free translation system.

Please replace the second full paragraph of page 10 with the following paragraph:

If desired, an [Ehrlichia] *Ehrlichia* polypeptide can be produced as a fusion protein, which can also contain other amino acid sequences, such as amino acid linkers or signal sequences, as well as ligands useful in protein purification, such as glutathione-S-transferase, histidine tag, and staphylococcal protein A. More than one [Ehrlichia] *Ehrlichia* polypeptide can be present in a fusion protein. If desired, various combinations of [Ehrlichia] *Ehrlichia* polypeptides from different [ehrilichia] *Ehrlichia* strains or isolates can be included in a fusion protein.

Please replace the third full paragraph of page 10 with the following paragraph:

A polypeptide of the invention can be synthesized such that it comprises several repeated [Ehrlichia] *Ehrlichia* polypeptides. This is a multimeric polypeptide. These

repeated polypeptides can comprise one specific polypeptide, *e.g.* the polypeptide shown in SEQ ID NO:1, repeated 2or more times. Alternatively, the repeated polypeptides can comprise one or more copies of a specific [Ehrlichia] *Ehrlichia* polypeptide along with one or more copies of another different [Ehrlichia] *Ehrlichia* polypeptide. A polypeptide of the invention can be combined or synthesized with one or more polypeptides, fragments of polypeptides, or full-length polypeptides. Preferably the one or more polypeptides are other polypeptides of the invention or other [Ehrlichia] *Ehrlichia* proteins.

Please replace the third full paragraph of page 11 with the following paragraph:

Various strains and isolates of *Ehrlichia canis* and *Ehrlichia chaffeensis* occur, and polypeptides of any of these strains and isolates can be used in the present invention. Nucleic acid and amino acid sequences of [Ehrlichia] *Ehrlichia* genes and polypeptides are known in the art. For example, several sequences of the *E. chaffeensis* OMP gene family and several sequences of the *E. canis* P30 gene family are disclosed in WO 99/13720.

Please replace the first full paragraph of page 12 with the following paragraph:

The methods comprise contacting a polypeptide of the invention with a test sample under conditions that allow a polypeptide/antibody complex to form. The formation of a complex between the polypeptide and [anti-Ehrlichia] anti-*Ehrlichia* antibodies in the sample is detected. In one embodiment of the invention, the polypeptide/antibody complex is detected when an indicator reagent, such as an enzyme, which is bound to the antibody, catalyzes a detectable reaction. Optionally, an indicator reagent comprising a signal generating compound can be applied to the

polypeptide/antibody complex under conditions that allow formation of a polypeptide/antibody/indicator complex. The polypeptide/antibody/ indicator complex is detected. Optionally, the polypeptide or antibody can be labeled with an indicator reagent prior to the formation of a polypeptide/antibody complex. The method can optionally comprise a positive or negative control.

Please replace the first full paragraph of page 13 with the following paragraph:

Polypeptides of the invention can be used to detect [anti-Ehrlichia] anti-*Ehrlichia* antibodies or antibody fragments in assays including, but not limited to enzyme linked immunosorbent assay (ELISA), western blot, IFA, radioimmunoassay (RIA), hemagglutination (HA), and fluorescence polarization immunoassay (FPIA). A preferred assay of the invention is the reversible flow chromatographic binding assay, for example a SNAP® assay. *See* U.S. Pat. No. 5,726,010.

Please replace the second full paragraph of page 13 with the following paragraph:

In one type of assay format, one or more polypeptides can be coated on a solid phase or substrate. A test sample suspected of containing [anti-Ehrlichia] anti-*Ehrlichia* antibodies is incubated with an indicator reagent comprising a signal generating compound conjugated to an antibody specific for [Ehrlichia] *Ehrlichia* for a time and under conditions sufficient to form antigen/antibody complexes of either antibodies of the test sample to the polypeptides of the solid phase or the indicator reagent compound conjugated to an antibody specific for [Ehrlichia] *Ehrlichia* to the polypeptides of the solid phase. The reduction in binding of the indicator reagent conjugated to an [anti-Ehrlichia] anti-*Ehrlichia* antibody to the solid phase can be quantitatively measured. A measurable reduction in the signal compared to the signal generated from a confirmed

negative [Ehrlichia] *Ehrlichia* test sample indicates the presence of [anti-Ehrlichia] anti-*Ehrlichia* antibody in the test sample. This type of assay can quantitate the amount of [anti-Ehrlichia] anti-*Ehrlichia* antibodies in a test sample.

Please replace the first full paragraph of page 14 with the following paragraph:

In another type of assay format, one or more polypeptides of the invention are coated onto a support or substrate. A polypeptide of the invention is conjugated to an indicator reagent and added to a test sample. This mixture is applied to the support or substrate. If [Ehrlichia] *Ehrlichia* antibodies are present in the test sample they will bind the polypeptide conjugated to an indicator reagent and to the polypeptide immobilized on the support. The polypeptide/antibody/indicator complex can then be detected. This type of assay can quantitate the amount of [anti-Ehrlichia] anti-*Ehrlichia* antibodies in a test sample.

Please replace the second full paragraph of page 15 with the following paragraph:

The methods of the invention can also indicate the amount or quantity of [anti-Ehrlichia] anti-*Ehrlichia* antibodies in a test sample. With many indicator reagents, such as enzymes, the amount of antibody present is proportional to the signal generated. Depending upon the type of test sample, it can be diluted with a suitable buffer reagent, concentrated, or contacted with a solid phase without any manipulation. For example, it usually is preferred to test serum or plasma samples which previously have been diluted, or concentrate specimens such as urine, in order to determine the presence and/or amount of antibody present.

Please replace the third full paragraph of page 15 with the following paragraph:

The invention further comprises assay kits for detecting [anti-Ehrlichia] anti-*Ehrlichia* antibodies in a sample. A kit comprises one or more polypeptides of the invention and means for determining binding of the polypeptide to [Ehrlichia] *Ehrlichia* antibodies in the sample. A kit can comprise a device containing one or more polypeptides of the invention and instructions for use of the one or more polypeptides for the identification of an [Ehrlichia] *Ehrlichia* infection in a mammal. The kit can also comprise packaging material comprising a label that indicates that the one or more polypeptides of the kit can be used for the identification of [Ehrlichia] *Ehrlichia* infection. Other components such as buffers, controls, and the like, known to those of ordinary skill in art, may be included in such test kits. The polypeptides, assays, and kits of the invention are useful, for example, in the diagnosis of individual cases of [Ehrlichia] *Ehrlichia* infection in a patient, as well as epidemiological studies of [Ehrlichia] *Ehrlichia* outbreaks.

Please replace the first full paragraph of page 16 with the following paragraph:

Polypeptides and assays of the invention can be combined with other polypeptides or assays to detect the presence of [Ehrlichia] *Ehrlichia* along with other organisms. For example, polypeptides and assays of the invention can be combined with reagents that detect heartworm and/or *Borrelia burgdorferi*.

Please replace the third full paragraph of page 16 with the following paragraph:

The antibodies or fragments thereof can be employed in assay systems, such as a reversible flow chromatographic binding assay, enzyme linked immunosorbent assay, western blot assay, or indirect immunofluorescense assay, to determine the presence, if any, of [Ehrlichia] *Ehrlichia* polypeptides in a test sample. In addition, these antibodies,

in particular monoclonal antibodies, can be bound to matrices similar to CNBr-activated Sepharose and used for the affinity purification of specific [Ehrlichia] *Ehrlichia* proteins from, for example, cell cultures or blood serum, such as to purify recombinant and native [Ehrlichia] *Ehrlichia* antigens and proteins. The monoclonal antibodies of the invention can also be used for the generation of chimeric antibodies for therapeutic use, or other similar applications.

Please replace the fourth full paragraph of page 16 with the following paragraph:

Monoclonal antibodies directed against [Ehrlichia] *Ehrlichia* epitopes can be produced by one skilled in the art. The general methodology for producing such antibodies is well-known and has been described in, for example, Kohler and Milstein, Nature 256:494 (1975) and reviewed in J. G. R. Hurrel, ed., Monoclonal Hybridoma Antibodies: Techniques and Applications, CRC Press Inc., Boca Raton, Fla. (1982), as well as that taught by L. T. Mimms *et al.*, *Virology* 176:604-619 (1990). Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus.

Please replace the second full paragraph of page 18 with the following paragraph:

The synthetic peptide SNAP® assay and native antigen SNAP® assay comprised an assay system similar to that described in U.S. Pat. No. 5,726,010. Briefly, a test sample is applied to a reverse flow chromatographic binding assay device and allowed to flow along and saturate a flow matrix. This facilitates sequential complex formation. That is, an [Ehrlichia] *Ehrlichia* antibody in the test sample binds first to an non-immobilized labeled specific binding reagent. In the case of the synthetic peptide

SNAP® assay the non-immobilized labeled specific binding reagent is a polypeptide of the invention conjugated to horseradish peroxidase. For the native antigen SNAP® assay the reagent comprises partially purified native antigens. This complex binds to an immobilized analyte capture reagent. For the synthetic peptide SNAP® assay the immobilized analyte capture reagent is one or more polypeptides of the invention conjugated to bovine serum albumin. For the native antigen SNAP® assay the capture reagent is partially purified native antigens. An absorbent reservoir is contacted with the saturated flow matrix, thereby reversing the fluid flow. Detector and wash solution is delivered to the flow matrix. The liquid reagents remove unbound sample and unbound labeled specific binding reagent and facilitate detection of analyte complexes at the location of the of the immobilized analyte capture reagent. The substrate used in these experiments was 3,3',5,5' tetramethylbenzidine (TMB).